Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

International Journal of Food Microbiology 101 (2005) 179–187

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Model for the combined effects of temperature, pH and sodium chloride concentration on survival of *Shigella flexneri* strain 5348 under aerobic conditions☆

Laura L. Zaika[a,*], John G. Phillips[b]

[a]*Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA*

[b]*North Atlantic Area Office, U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA*

Received 5 July 2004; received in revised form 8 October 2004; accepted 8 November 2004

Abstract

Shigella is recognized as a major foodborne pathogen; however, relatively few studies have been reported on its growth and survival characteristics, particularly under conditions relevant to food. A fractional factorial design was used to measure the effects and interactions of temperature (4–37 °C), pH (2–6) and NaCl (0.5–9%) on survival kinetics of *Shigella flexneri* strain 5348 in BHI broth. Stationary-phase cells were inoculated into sterile media to give initial populations of 6–7 $\log_{10}$ CFU/ml and bacterial populations were determined periodically by aerobic plate counts. A total of 267 cultures, representing 83 variable combinations of temperature, pH and NaCl concentration, were analyzed. Survivor curves were fitted from plate count data by means of a two-phase linear model to determine lag times and slopes of the curves, from which decimal reduction times (D-values) and times to a 4-$\log_{10}$ inactivation (t_{4D}) were calculated. Second order response surface models in terms of temperature, initial pH and NaCl concentration were obtained for the inactivation kinetics parameters of *S. flexneri* using regression analysis. The use of $\log_{10}$ transformation of the inactivation kinetics parameters yielded models with R^2 values of >0.8. These models can provide an estimate of *Shigella* inactivation. The data obtained suggest that *Shigella* is resistant to acid and salt and that low pH foods stored at low temperatures may serve as vehicles for gastrointestinal illness.
Published by Elsevier B.V.

Keywords: *Shigella flexneri*; Response surface models; Inactivation kinetics; Temperature; pH; NaCl

☆ Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Corresponding author. Tel.: +1 215 233 6614; fax: +1 215 233 6434.
E-mail address: jphilips@errc.ars.usda.gov (L.L. Zaika).

0168-1605/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2004.11.004

1. Introduction

Shigella is a major cause of gastrointestinal illness throughout the world. In the United States *Shigella* ranks third among bacterial foodborne pathogens (after *Campylobacter* and *Salmonella*) in the number of illness cases, according to surveys by the Centers for Disease Control and Prevention (Mead et al., 1999). The incidence of infection with *Shigella* is estimated at 448,000 cases per year, with 20% of these cases being due to foodborne transmission of the pathogen. Epidemiological studies frequently implicate infected food handlers and fecally contaminated water as the sources of contamination in shigellosis outbreaks. In spite of these statistics, relatively few studies have been conducted to determine the ability of *Shigella* to grow and/or survive under conditions relevant to food. *Shigella* spp. grew readily when inoculated into foods such as shredded cabbage (Satchell et al., 1990), sliced papaya and jicama (Fernandez Escartin et al., 1989) and various sterile foods (Islam et al., 1993; Zaika and Scullen, 1996). The organism survived for extended periods of time in a variety of foods, even in salty foods (Siegmund, 1960; Taylor and Nakamura, 1964) and in acidic foods at <pH 4.6 (Siegmund, 1960; Taylor and Nakamura, 1964; Rafii and Lunsford, 1997).

Predictive models, based on microbial growth or inactivation kinetics, are useful as a means of estimating the ability of a microorganism to grow or survive under conditions relevant to the formulation, processing or storage of food. For model development, detailed information is needed on the response of the microorganism to a variety of factors and their interactions, such as temperature, pH, salt levels, food additives, atmosphere and others. We have developed response surface models which describe the effects and interactions of temperature, initial pH, sodium chloride and sodium nitrite concentrations on growth of *Shigella flexneri* strain 5348 in culture media under both aerobic (Zaika et al., 1998) and anaerobic conditions (Zaika et al., 1994). We also determined that good agreement was obtained between *S. flexneri* growth kinetics values calculated with the aid of the aerobic model and those observed in foods (Zaika et al., 1998). However, models for inactivation of *Shigella* have not been reported. As part of our objective to develop a model for inactivation of *Shigella* in food, we have studied the survival characteristics of *S. flexneri* in a microbiological medium under various combinations of temperature, pH and sodium chloride concentration and obtained inactivation kinetics data for conditions that do not support growth (Zaika, 2001, 2002a). The objective of the present work was to use these and additional inactivation kinetics data to develop a model to describe the inactivation of *S. flexneri* as a function of temperature, initial pH and NaCl concentration.

2. Materials and methods

2.1. Microorganism

S. flexneri strain 5348 (obtained from Dr. David W. Niesel, University of Texas Medical Branch, Galveston, TX) was used throughout the study. This strain has been studied extensively in our laboratory and was used for the development of growth models for *S. flexneri* (Zaika et al., 1994, 1998). A stock culture of strain 5348 was stored at −70 °C in cryovials (Nalgene) containing BHI (Difco Laboratories, Detroit, MI) broth supplemented with 10% (v/v) glycerol. To prepare the inoculum for each trial, a 0.1-ml portion of the thawed stock culture was added to 10 ml of BHI broth and incubated without shaking for 18–24 h at 37 °C. The resulting stationary phase cells were used to inoculate test media.

2.2. Experimental design

An incomplete factorial design was used to assess the effects and interactions of temperature (4, 12, 19, 28, 37 °C), initial pH (2, 3, 4, 5, 6) and NaCl (0.5, 2, 4, 5, 6, 7, 8, 9%) on inactivation of *S. flexneri* in BHI broth. The number of replicate cultures tested for each variable combination is given in Tables 1 and 2.

2.3. Media

BHI broth, supplemented with salt to give final concentrations of 0.5, 2, 4, 5, 6, 7, 8 or 9% NaCl (w/v), was adjusted to pH 2, 3, 4, 5 or 6 with 6 N HCl. Portions (100 ml) were dispensed into 250-ml Erlenmeyer flasks capped with foam plugs and sterilized by autoclaving. Autoclaving of the media

Table 1
Conditions of temperature, pH and NaCl concentration in *S. flexneri* cultures that resulted in growth or a poor fit to the primary model

T °C	pH	NaCl %	n[a]	
12	5	2	2	DPF[b]
12	5	4	2	DPF
12	6	0.5	2	GR[c]
12	6	2	2	GR
19	5	0.5	2	GR
19	5	2	2	GR
19	5	4	2	GR
19	6	4	2	GR
19	6	5	4	GR
19	6	6	4	GR
28	5	0.5	2	GR
28	5	2	2	GR
28	5	4	2	GR
28	6	5	2	GR
28	6	6	4	GR
28	6	7	4	GR
37	5	0.5	1	GR
37	5	2	1	GR
37	6	0.5	2	GR
37	6	2	2	GR
37	6	4	2	GR
37	6	6	2	DPF

[a] n=number of replicate cultures.
[b] DPF=decline, poor fit.
[c] GR=growth.

did not result in significant changes in pH (±0.1 pH unit). An Orion Research ionanalyzer/501 (Orion Research, Inc., Boston, MA) equipped with a sealed combination electrode with silver/silver chloride reference, 0–14 pH (VWR Scientific, San Francisco, CA) was used for pH determination.

2.4. Culture conditions

Portions (1-ml) of the inoculum were added to the media to give an initial population level of 6–7 $\log_{10}$ CFU/ml. The media were incubated on a rotary shaker (150 rev./min) at the desired temperature (4, 12, 19, 28 or 37 °C) until bacterial populations reached undetectable levels (<1.3 $\log_{10}$ CFU/ml) by direct plating.

2.5. Determination of microbial populations

Bacterial populations were determined immediately after inoculation (initial count) and at appropriate time intervals by surface-plating cultures, or dilutions thereof prepared in 0.1% peptone water, onto duplicate plates of tryptose agar (Difco) using a Spiral Plater (Model D; Spiral System Instruments, Bethesda, MD). The plates were incubated for 48 h at 37 °C and the colonies were counted. Populations in cultures showing visible growth were determined by the same procedure.

2.6. Curve fitting

Survivor curves were generated from the experimental data (population vs. time) using the two-phase linear model of Buchanan et al. (1993). This model (Table 3) allows for the presence of a lag period before the beginning of the exponential decline in bacterial population density. The curves were fitted using ABACUS, a non-linear regression program that employs a Gauss–Newton iterative procedure (Damert, 1994). A population decrease of ≥3 $\log_{10}$ CFU/ml and a minimum of 7 experimental data points were designated as acceptable to generate valid survivor curves.

2.7. Statistical analyses

Second order response surface models in terms of temperature, pH and NaCl concentration were calculated for the *S. flexneri* inactivation data using least squares analysis of PROC GLM of the SAS system (SAS Institute, Inc., 1989). The regression analysis was performed on the untransformed inactivation parameters, t_L, s, D-value and t_{4D}, and on the logarithmic transformations of the parameters, $\log_{10}(t_L)$, $\log_{10}(s)$, $\log_{10}$ (D-value) and $\log_{10}(t_{4D})$.

3. Results and discussion

A total of 267 cultures of *S. flexneri*, representing 83 variable combinations of temperature, initial pH and NaCl concentration, were analyzed. This total includes 196 observations obtained previously (Zaika, 2001, 2002a) Survivor curves were fitted from the experimental data using a two-phase linear model (Buchanan et al., 1993) to obtain lag times, t_L, and slopes of the curves, s, from which decimal reduction times, D-values, and times to a 4-$\log_{10}$ (99.99%) inactivation, t_{4D}, were calculated. This

Table 2
Inactivation kinetics values[a] for *S. flexneri* incubated aerobically in BHI broth under various combinations of temperature, pH and NaCl concentrations

T °C	pH	NaCl %	n[b]	N_o	t_L	s	D	t_{4D}
				$\log_{10}$ CFU/ml	h	($\log_{10}$ CFU/ml)/h	h	h
4	2	0.5	4	6.87	2.63	−0.3254	3.20	15.25
4	3	0.5	4	6.75	234.26	−0.0149	74.54	532.42
4	4	0.5	3	6.80	918.35	−0.0051	208.61	1752.79
4	5	0.5	4	7.10		0.0000		
12	2	0.5	2	7.22	11.90	−0.0781	12.81	63.14
12	3	0.5	8	7.06	174.12	−0.0163	61.95	421.88
12	3	2	2	6.96	79.16	−0.0140	71.62	365.76
12	3	4	2	7.12	10.64	−0.0131	76.27	315.70
12	3	6	2	7.05	0.00	−0.0102	97.88	391.52
12	4	0.5	7	6.51	621.31	−0.0053	208.30	1454.63
12	4	2	4	6.64	989.35	−0.0068	155.10	1609.77
12	4	4	4	6.64	406.00	−0.0050	203.34	1219.37
12	4	6	4	6.67	319.80	−0.0037	270.66	1402.45
12	5	0.5	3	6.21	1449.59	−0.0022	470.68	3332.32
12	5	6	2	6.76	809.67	−0.0018	566.90	3077.27
12	6	6	2	6.50	0.00	−0.0009	1091.49	4365.96
19	2	0.5	3	7.26	1.74	−0.2256	5.32	23.01
19	3	0.5	5	6.69	105.21	−0.0356	28.90	220.83
19	3	2	2	6.76	19.51	−0.0258	38.71	174.35
19	3	4	2	6.62	0.00	−0.0248	40.31	161.22
19	3	6	2	6.43	0.00	−0.0250	39.92	159.68
19	4	0.5	11	6.85	276.86	−0.0219	51.79	484.02
19	4	2	7	6.78	298.22	−0.0137	107.20	726.90
19	4	4	7	6.77	120.71	−0.0114	120.38	602.25
19	4	6	8	6.63	22.42	−0.0131	116.88	489.91
19	4	8	4	6.61	0.00	−0.0579	25.23	100.90
19	5	5	3	6.78	274.40	−0.0063	183.97	1010.00
19	5	6	3	6.48	0.00	−0.0053	192.19	766.11
19	5	7	2	7.00	76.26	−0.0044	232.48	1006.16
19	5	8	3	6.92	0.00	−0.0089	122.99	491.96
19	5	9	2	7.00	6.06	−0.0139	88.87	401.54
19	6	7	1	6.30	2.00	−0.0129	77.46	311.84
19	6	8	3	6.50	0.00	−0.0042	267.41	1069.64
19	6	9	2	6.98	0.00	−0.0032	314.23	1256.90
28	2	0.5	2	6.82	0.53	−0.8923	1.13	5.05
28	3	0.5	4	6.97	13.77	−0.0518	20.23	94.68
28	4	0.5	9	6.84	114.34	−0.0278	38.01	266.39
28	4	2	4	6.70	144.52	−0.0232	45.56	326.76
28	4	4	4	6.58	129.25	−0.0220	47.46	319.07
28	4	6	2	6.80	0.00	−0.0115	87.28	349.14
28	5	5	3	6.58	6.21	−0.0122	82.42	335.88
28	5	6	6	6.42	0.00	−0.0125	86.19	344.76
28	5	7	4	6.35	0.00	−0.0107	99.23	396.91
28	5	8	4	6.43	0.00	−0.0152	72.16	288.63
28	5	9	2	6.74	0.00	−0.0066	152.66	610.66
28	6	8	4	6.50	15.79	−0.0078	129.94	560.53
28	6	9	2	5.83	129.62	−0.0158	63.28	382.74
37	2	0.5	2	6.98	0.21	−3.9999	0.25	1.21
37	3	0.5	5	6.77	3.89	−0.6504	1.59	10.24
37	3	2	1	6.55	1.94	−0.2850	3.51	15.98

Table 2 (*continued*)

T °C	pH	NaCl %	n[b]	N_o	t_L	s	D	t_{4D}
				$\log_{10}$ CFU/ml	h	($\log_{10}$ CFU/ml)/h	h	h
37	3	4	2	6.39	0.00	−0.4148	2.42	9.66
37	3	6	2	6.11	0.00	−0.4108	2.47	9.88
37	4	0.5	5	7.09	25.45	−0.1049	9.81	64.66
37	4	2	2	6.73	30.42	−0.0694	14.56	88.68
37	4	4	2	6.70	16.99	−0.0462	21.66	103.58
37	4	6	2	6.78	0.00	−0.0393	25.64	102.52
37	5	4	1	6.44	0.00	−0.0190	52.74	210.96
37	5	6	1	6.52	0.00	−0.0170	58.84	235.36
37	6	7	4	7.08	0.00	−0.1160	28.19	112.75
37	6	8	4	7.20	0.00	−0.0442	22.71	90.84
37	6	9	4	7.02	0.00	−0.0463	21.74	86.97

[a] For abbreviations, see Table 3.
[b] Number of replicate cultures.

model was used successfully in our previous work to describe the inactivation of *S. flexneri* (Zaika, 2001, 2002a,b). To obtain a data set suitable for the development of inactivation models, a total of 53 observations were excluded because of bacterial growth or because, although the bacterial populations declined, a poor fit of the model was obtained. Forty-four observations, representing 19 variable combinations, were excluded due to bacterial growth and 6 observations, representing 3 variable combinations, were excluded due to a poor fit to the primary model (Table 1). In addition, growth was observed in 2 of 5 cultures for the variable combination of 12 °C, pH 5 and 0.5% NaCl. Also, in one of 2 replicates for the variable combination of 19 °C, pH 6 and 7% NaCl, although the bacterial population declined, a poor fit to the model was obtained. Thus, 214 observations, representing 61 variable combinations were available for the development of an inactivation model for *S. flexneri*. These data are summarized in Table 2.

Table 3
Model to mathematically represent individual survivor curves[a]

	Two-phase linear inactivation model
Phase 1: $N=N_o$ [$t<t_L$]	
Phase 2: $N=N_o+s(t-t_L)$ [$t>t_L$]	
Where:	
N=Log_{10} count of bacteria at time t	[Log_{10} (CFU/ml)]
N_o=Log_{10} count of bacteria at time t=0	[Log_{10} (CFU/ml)]
s=Slope of survivor curve	[Log_{10} (CFU/ml)/h]
t=Time	[h]
t_L=Duration of lag period prior to commencement of inactivation	[h]

[a] D-values were calculated by taking the negative reciprocal of the "s" term, and "time to a 4−D (99.99%) inactivation" was calculated using the following equation: $t_{4D}=t_L+(4\times D)$.

Survival of *S. flexneri* strain 5348 increased with decreasing temperature, increasing pH and decreasing NaCl concentration. Growth occurred at pH 5 and 6 at temperatures ≥12 °C, but was highly dependent on the NaCl concentration in the medium. At 12, 19, 28 and 37 °C, growth was observed in broth of pH 5 containing 0.5, ≤4, ≤4 and ≤2% NaCl, respectively, and in broth of pH 6 containing ≤2, ≤6, ≤7 and ≤4% NaCl, respectively. Growth did not occur at 4 °C or at ≤pH 4. For the variable combination of 4 °C, pH 5 and 0.5% NaCl, inactivation kinetics parameters were not calculated since the bacterial population decreased only 0.5–1.5 $\log_{10}$ CFU/ml in 75 days; thus, the slope of the survivor curve, s, was assigned the value of zero (Table 2). Although our previous investigations confirmed that NaCl had a major effect on the growth of *S. flexneri* (Zaika et al., 1994, 1998; Zaika, 2002a), in the present study survival of the organism was affected to a lesser extent by NaCl. Lag times (t_L) decreased with increasing NaCl concentration; however, the effect on decimal reduction times (D-values) and times to a 4-$\log_{10}$ inactivation (t_{4D}) was less pronounced than on lag times (Table 2). Published reports indicate that *Shigella* spp. may survive in salty foods for long periods of time, depending on storage temperature. For example, the average survival times for strains of *S. flexneri* and *Shigella sonnei* at 22 °C

in sauerkraut (pH 3.9), in herring brine containing 25% NaCl (pH 4.1) and in meat salad (pH 5.0) were 16, 3 and 32 days, respectively, and at 4 °C the survival times were 22, 21 and 52 days, respectively (Siegmund, 1960).

Second order response surface models describing the effects and interactions of temperature, initial pH and NaCl concentration on inactivation of *S. flexneri* were generated using the untransformed kinetics values as well as their logarithmic transformations to stabilize the variances (Table 4). The logarithmic transformation greatly stabilized the variance for t_L, D-value and t_{4D} and, to a lesser extent, the variance for s. Examination of the R^2 values for the models obtained (Table 5) indicated that the use of the $\log_{10}$ transformations for all four inactivation kinetics parameters resulted in much better fits (R^2>0.8) than without logarithmic transformation. Although the D-value is the negative reciprocal of the slope of the survivor curve, s, there is not much similarity in the models for D-value and s. The correlation coefficient between D-value and s is 0.165. Although this value is significantly non-zero, it is not especially large (i.e., close to 1). The difficulty in modeling lag time has been recognized by other workers (Buchanan et al., 1994; Geeraerd et al., 2000; Swinnen et al., 2004). In our work, 73 observations, representing 22 variable combinations (Table 2) were not included into the model for $\log_{10}$ (t_L) since no lag time was observed. This represents a significant portion of the data set. The highest R^2 value, 0.887, was obtained for the $\log_{10}$ (t_{4D}) model, which takes into account both the lag time and the slope of the inactivation curve.

Table 4
Second order response surface models for describing the effects and interactions of temperature (T, °C), initial pH (P) and sodium chloride concentration (S, %) on inactivation of *S. flexneri*

$t_L = -538.2 - 5.57T + 308.3P + 6.29S - 10.85TP + 3.94TS - 45.49PS + 0.4875T^2 + 32.064P^2 + 5.791S^2$

$s = -0.983 - 0.0467T + 0.583P + 0.085S + 0.0186TP + 0.00004TS - 0.0263PS - 0.000872T^2 - 0.08963P^2 + 0.001767S^2$

$D = -19.12 + 4.00T - 75.9P + 71.83S - 6.507TP - 0.329TS - 10.758PS + 0.30117T^2 + 44.724P^2 - 3.082S^2$

$t_{4D} = -614.94 + 10.57T + 4.48P + 2921.4S - 36.871TP + 2.605TS - 88.43PS + 1.6893T^2 + 210.955P^2 - 6.333S^2$

$\log_{10}(t_L) = -4.45 - 0.0087T + 3.408P - 0.3618S - 0.00327TP + 0.00561TS + 0.0709PS - 0.0008456T^2 - 0.3711P^2 - 0.0242S^2$

$\log_{10}(s) = +1.65 - 0.0039T - 1.557P - 0.0205S + 0.0028TP - 0.00137TS - 0.018PS + 0.000931T^2 + 0.1385P^2 + 0.01575S^2$

$\log_{10}(D) = -1.48 - 0.0007T + 1.46P + 0.04318S - 0.00163TP + 0.001245TS + 0.01364PS - 0.0009061T^2 - 0.12683P^2 - 0.01631S^2$

$\log_{10}(t_{4D}) = -1.297 - 0.00213T + 1.83P - 0.05415S - 0.002278TP + 0.001672TS + 0.02003PS - 0.00090T^2 - 0.16686P^2 - 0.0136S^2$

Table 5
Comparison of the goodness of fit[a] of second order response surface models for inactivation of *S. flexneri*

Model	R^2	Adj. R^2	n[b]
t_L	0.667	0.722	210
s	0.528	0.532	214
D	0.713	0.758	210
t_{4D}	0.789	0.820	210
$\log_{10}$ (t_L)	0.839	0.890	137
$\log_{10}$ (s)	0.858	0.904	210
$\log_{10}$ (D)	0.868	0.907	210
$\log_{10}$ (t_{4D})	0.887	0.919	210

[a] Adjusted R^2=R^2/Max R^2 (Draper and Smith, 1981).
[b] No. of observations; t_L=0 responses were treated as missing values.

The $\log_{10}$ transformation-based models were evaluated by comparing the observed inactivation kinetics values to those calculated using the models (Fig. 1). In general, good agreement was obtained for all four models. In all cases, the points falling outside the limits of the models were represented by 15 variable combinations, 8 of which included pH 5 or 6. This was not surprising, since, at pH 5 and 6, difficulties were encountered in obtaining satisfactory inactivation curves at NaCl levels in the region of transition from growth to inactivation (Zaika, 2002a). Thus, it is possible that an initial decline in *S. flexneri* population at the higher concentrations of NaCl may be followed by growth, and this decline may be more rapid than at NaCl concentrations that do not permit growth (Zaika, 2002a). Thus, these models may be useful in estimating the inactivation of *S. flexneri* under conditions at <pH 5.

There are few, if any, published reports on inactivation kinetics of *Shigella* that could be used to evaluate the models we obtained. Published studies on the growth and survival of *Shigella* spp. have been reviewed (ICMSF, 1996). Although a number of studies on the survival characteristics of *Shigella* as influenced by temperature, pH and NaCl concentration have been reported, sufficient details are not available to permit calculation of inactivation

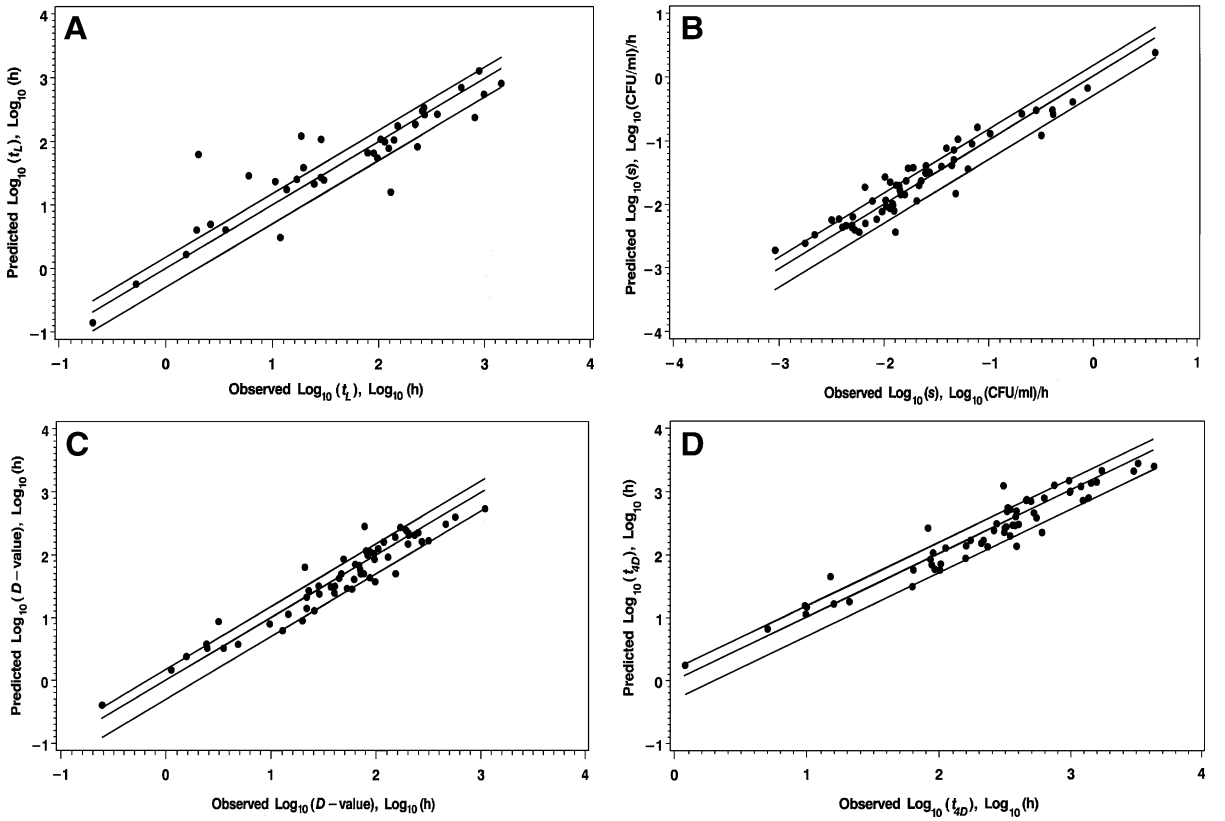

Fig. 1. Comparison of observed inactivation kinetics parameters with those calculated using the models, (A) Log_{10} (t_L), (B) Log_{10} (s), (C) Log_{10} (D-value) and (D) Log_{10} (t_{4D}). Predictions for variable combinations corresponding to t_L=0 and s=0 are not included in the figures. The center line represents the line of identity and the two flanking lines are ±50% of the observed value.

kinetics parameters from the data presented. Mossel and de Bruin (1960) studied the survival of *Enterobacteriaceae*, including *S. sonnei*, in acid liquid foods stored at 5 and 24 °C and reported the observed times to a four-decimal reduction in bacterial population (t_{4D}). We compared the reported t_{4D} values for *S. sonnei* with those predicted with the log_{10} (t_{4D}) model. The NaCl concentrations were not stated; therefore, we used 0.5% NaCl for all calculations (Table 6).

The predicted inactivation times for both temperatures agreed well with those observed by Mossel and de Bruin (1960), particularly for apple, orange and lemon juices. Although some workers (Stelzner and Urbach, 1969; Fehlhaber, 1981) reported that *S. sonnei* survived better in foods than did *S. flexneri*, others (Taylor and Nakamura, 1964; Nass, 1965) observed that the survival rates of the two microorganisms were comparable. Previously, we studied the inactivation of *S. flexneri* in BHI broth, pH 4, containing 0.04 M organic acids, at 4, 19, 28 and

Table 6
Comparison of inactivation of *S. sonnei* observed[a] in fruit juices with inactivation predicted by model for $Log_{10}(t_{4D})$

	pH[b]	T °C	Observed	Predicted
			t_{4D} days	t_{4D} days
Tomato juice	4.0	5	38–49	80.9
(pH 3.9–4.1)	4.0	24	7–10	16.4
Apple juice	3.0	5	5–35	17.7
(pH 3.0–3.1)	3.0	24	2–10	4.0
Orange juice	3.3	5	10–35	30.2
(pH 3.1–3.5)	3.3	24	4–7	6.6
Lemon juice	2.4	5	1	4.9
(pH 2.1–2.6)	2.4	24	1	1.2

[a] Data from Mossel and de Bruin (1960).
[b] Average of pH values reported by Mossel and de Bruin (1960).

37 °C (Zaika, 2002b). Good agreement was obtained between the observed t_{4D} values and those predicted by the $\log_{10}$ (t_{4D}) model for the control culture (HCl only; not included in model development) and for cultures containing citric, malic or tartaric acid, while the observed t_{4D} values for cultures containing acetic and lactic acids were considerably smaller than the predicted values (Table 7). This is consistent with generally recognized observations that acetic and lactic acids exert a strong inhibitory effect on pathogenic bacteria, among them *Shigella* (Mossel and de Bruin, 1960; Zaika, 2002b).

It should be noted that in the development of these models a standard procedure was used for preparation of the inoculum, namely growth in BHI broth at 37 °C. Conditions under which the inoculum is grown may subsequently affect the ability of the organism to grow or survive under adverse conditions. Acid adaptation of various bacteria is generally recognized. Recently, Tetteh and Beuchat (2003) studied the effect of acid adaptation and acid shock on the survival, growth and inactivation of *S. flexneri* 2a in tryptic soy broth (TSB) as affected by pH (7.3, 5.5, 5.0, 4.5, 4.0 and 3.5) during incubation at 4, 12, 21, 30 and 48 °C for up to 144 h. Unadapted cells were grown in TSB, acid-adapted cells were grown in TSB supplemented with 1% glucose and acid-shocked cells were prepared by briefly exposing unadapted cells to TSB adjusted to pH 4.5 with lactic acid. Tetteh and Beuchat (2003) concluded that prior exposure of *S. flexneri* cells to an acidic environment rendered them more resistant to extremes in acid and temperature. Although the general trend in the response of *S. flexneri* to conditions of pH and temperature observed in the present study was similar to that observed by Tetteh and Beuchat (2003), a direct comparison of the results is not valid since these workers used lactic acid to acidify their test media, thus introducing an additional factor into their experimental design. The effect of lactic acid concentration would have to be considered, in addition to pH. Our experiments showed that *S. flexneri* survived to a significantly lesser extent at 4, 19, 28 and 37 °C in BHI containing 0.04 M lactic acid adjusted to pH 4 with HCl than in the control medium, BHI adjusted to pH 4 with HCl (Table 7). Since the infective dose of *Shigella* may be as low as 10 to 500 organisms (DuPont et al., 1989), the results of the present study suggest that foods of pH≤5, even those containing moderate levels of NaCl (2 to 4%), stored at or below room temperature may permit the survival of the bacterium over long periods of time in sufficient numbers to cause gastrointestinal illness.

The response surface models obtained in this study, particularly the one for $\log_{10}$ (t_{4D}), are intended to be used as a means of estimating survival of *S. flexneri* in foods under conditions within the boundaries of the range of the variables studied. The models are based on only three variables: temperature, pH and sodium chloride concentration. Other factors may have a significant impact on the survival of the organism and would have to be considered in future model development. These models will be included in the next edition of the USDA Pathogen Modeling Program (www.arserrc.gov/mfs/pathogen.htm) and the survival data will be incorporated into COMBASE (www.combase.cc).

Table 7
Comparison of inactivation of *S. flexneri* observed[a] in BHI broth[b] containing 0.04 M organic acids, adjusted to pH 4, with inactivation predicted by the model for $\text{Log}_{10}(t_{4D})$

T °C	Observed						Predicted
	t_{4D} h						t_{4D} h
	HCl[c]	Acetic acid	Citric acid	Lactic acid	Malic acid	Tartaric acid	
4	2606.8	1497.6	1904.6	1346.3	1725.6	2133.8	2026.7
19	578.2	454.7	476.1	408.4	478.5	501.4	692.1
28	231.5	121.3	137.4	101.7	155.4	166.5	232.2
37	64.5	46.8	50.1	34.4	58.4	51.8	55.7

[a] Data from Zaika (2002b).
[b] BHI broth contains 0.5% NaCl.
[c] BHI broth adjusted to pH 4 with HCl, control.

Acknowledgments

We thank Joseph S. Fanelli, Kelly Hassell and Marangeli Osoria for technical assistance in conducting the bacterial survival studies.

References

Buchanan, R.L., Golden, M.H., Whiting, R.C., 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. Journal of Food Protection 56, 474–478, 484.

Buchanan, R.L., Golden, M.H., Whiting, R.C., Phillips, J.G., Smith, J.L., 1994. Non-thermal inactivation models for *Listeria monocytogenes*. Journal of Food Science 59, 179–188.

Damert, W., 1994. ABACUS: Interactive Program for Nonlinear Regression Analysis. QCPE Bulletin, vol. 14. Indiana University, Bloomington, Ind, p. 61.

Draper, N.R., Smith, H., 1981. Applied Regression Analysis, 2nd ed. John Wiley & Sons, Inc., New York, NY, pp. 40–42.

DuPont, H.L., Levine, M.M., Hornick, R.B., Formal, S.B., 1989. Inoculum size in shigellosis and implications for expected mode of transmission. Journal of Infectious Diseases 159, 1126–1128.

Fehlhaber, K., 1981. Untersuchungen über das Verhalten von Shigellen in milchwirtschaftlich eingesetzten microbiellen Kulturen. Die Nahrung 25, 921–928.

Fernandez Escartin, E., Castillo Ayala, A., Saldana Lozano, J., 1989. Survival and growth of *Salmonella* and *Shigella* on sliced fresh fruit. Journal of Food Protection 52, 471–472.

Geeraerd, A.H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe microbial inactivation during mild heat treatment. International Journal of Food Microbiology 59, 185–209.

ICMSF, 1996. Microorganisms in Foods 5: Characteristics of Microbial Pathogens. In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Blackie Academic and Professional, London, pp. 280–298. Ch. 16.

Islam, M.S., Hasan, M.K., Khan, S.I., 1993. Growth and survival of *Shigella flexneri* in common Bangladeshi foods under various conditions of time and temperature. Applied and Environmental Microbiology 59, 652–654.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerging Infectious Diseases 5, 607–625.

Mossel, D.A.A., de Bruin, A.S., 1960. The survival of *Enterobacteriaceae* in acid liquid foods stored at different temperatures. Annales de l'Institut Pasteur de Lille 11, 65–72.

Nass, W., 1965. Untersuchungen über die Lebensdauer von *Shigella flexneri* und *Shigella sonnei* in künstlich infizierter Milch und in Milchprodukten. Archiv für Hygiene und Bakteriologie 149, 635–642.

Rafii, F., Lunsford, P., 1997. Survival and detection of *Shigella flexneri* in vegetables and commercially prepared salads. Journal of AOAC International 80, 1191–1197.

SAS Institute, Inc., 1989. SAS/STAT Users' Guide, Version 6, 4th ed., vol. 2. SAS Institute, Inc., Cary, NC, pp. 871–996.

Satchell, F.B., Stephenson, P., Andrews, W.H., Estela, L., Allen, G., 1990. The survival of *Shigella sonnei* in shredded cabbage. Journal of Food Protection 53, 558–563.

Siegmund, I., 1960. Untersuchungen über die Lebensdauer von Salmonellen und Shigellen in verschiedenartigen Lebensmitteln. Archiv für Hygiene und Bakteriologie 144, 550–563.

Stelzner, A., Urbach, H., 1969. Zur Frage der Haltbarkeit von Shigellen am Fleisch. Archiv für Hygiene und Bakteriologie 153, 532–537.

Swinnen, I.A.M., Bernaerts, K., Dens, E.J.J., Geeraerd, A.H., Van Impe, J.F., 2004. Predictive modelling of the microbial lag phase: a review. International Journal of Food Microbiology 94, 137–159.

Taylor, B.C., Nakamura, M., 1964. Survival of Shigellae in food. Journal of Hygiene 62, 303–311 (Cambridge).

Tetteh, G.L., Beuchat, L.R., 2003. Survival, growth, and inactivation of acid-stressed *Shigella flexneri* as affected by pH and temperature. International Journal of Food Microbiology 87, 131–138.

Zaika, L.L., 2001. The effect of temperature and low pH on survival of *Shigella flexneri* in broth. Journal of Food Protection 64, 1162–1165.

Zaika, L.L., 2002a. Effect of NaCl on survival of *Shigella flexneri* in broth as affected by temperature and pH. Journal of Food Protection 65, 774–779.

Zaika, L.L., 2002b. Effect of organic acids and temperature on survival of *Shigella flexneri* in broth at pH 4. Journal of Food Protection 65, 1417–1421.

Zaika, L.L., Scullen, O.J., 1996. Growth of *Shigella flexneri* in foods: comparison of observed and predicted growth kinetics parameters. International Journal of Food Microbiology 32, 91–102.

Zaika, L.L., Moulden, E., Weimer, L., Phillips, J.G., Buchanan, R.L., 1994. Model for the combined effects of temperature, initial pH, sodium chloride and sodium nitrite concentrations on anaerobic growth of *Shigella flexneri*. International Journal of Food Microbiology 23, 345–358.

Zaika, L.L., Phillips, J.G., Fanelli, J.S., Scullen, O.J., 1998. Revised model for aerobic growth of *Shigella flexneri* to extend the validity of predictions at temperatures between 10 and 19 °C. International Journal of Food Microbiology 41, 9–19.